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Using Mass Spectrometry for Drug Metabolism Studies

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Using Mass Spectrometry for Drug Metabolism Studies, edited by Walter A. Korfmacher, is a timely testament showing the availability of robust mass spectrometers, rugged chromatographic methods, sample preparation, and sample introduction techniques for improving all phases of drug discovery and development in an effort to produce safer and efficacious drugs. This book is clear and concise, written in a manner that is not overwhelming for a novice mass spectrometrist and not too simplistic for a seasoned mass spectrometrist. The book, divided into twelve chapters and authored by leaders in the field, eloquently conveys the art of mass spectrometry as it applies to drug metabolism studies and the quest to discover and develop new, safer, and efficacious medicines.

Chapter 1, entitled "Bioanalytical Assays in a Drug Discovery Environment", is authored by the editor. The focus of this chapter is on the use of LC-MS/MS for discovery stage drug-metabolism applications, especially for analysis of in vivo pharmacokinetic (PK) samples. Applications provided in this chapter underscore the need for faster liquid chromatography (LC)-tandem mass spectrometry (MS/MS) assays that can provide high quality data in a high throughput manner to impact the timelines and reduce the cost of bringing safe and effective drugs to patients. All aspects of LC-MS/MS assays and ways to improve throughput are discussed and include the following: (1) sample collection techniques, (2) sample preparation/processing techniques, and (3) various methods to maximize MS assay time. Although covered in detail in latter chapters, Chapter 1 also introduces the reader to LC-MS/MS assay development issues related to interferences from drug metabolites and background matrix ions/matrix ion suppression. For reference, a list of putative metabolites that could potentially interfere with assay through coelution and/or in-source fragmentation is provided. Additionally, topics introduced include higher mass resolution triple quadrupole and quadrupole linear ion trap mass spectrometers, atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), chip-based nanospray ion sources, automated MS/MS method development and metabolite identification software, and the potential utility of the above mentioned techniques in quantitative bioanalysis of drugs.

In the Current Practices section, the major steps in the discovery bioanalytical process including animal dosing, sample tracking, standard-curve preparation, sample preparation, LC-MS/MS assay development, data processing and PK report preparation, and common industry practices for streamlining these steps are very well presented. This section also provides the reader with a series of stages through which compounds must go before being recommended for full development. These stages include chemistry/synthesis, biology-high throughput screen, in vitro stability screen, caco-2 absorption screen, P450 enzyme inhibition screen, oral dose-rat PK screen (Level I), oral/IV dose rat/dog/monkey PK studies (Level II), discovery metabolite ID studies, special PK studies (Level III), and development studies (Level IV). LC-MS/MS assays involved in the latter four stages are categorized into four levels, and rules associated with these assays are tabulated and described in detail. This section will be of significance to those who want to establish the stage at which an LC-MS/MS assay without any quality control (QC) samples and two-point standard curve is sufficient to test a large number of compounds, and at which stage it will be necessary to comply with good laboratory practice (GLP) and follow other guidelines set forth by regulatory agencies such as the food and drug administration (FDA).

Chapter 2, "Drug Metabolism In Vitro and In Vivo Results: How Do These Data Support Drug Discovery?" is written by Thomas N. Thompson. This chapter serves as a very good review of overall drug metabolism and pharmacokinetics (DMPK) for any newcomer to the field, but fails to make a strong connection on how mass spectrometry measurements can be used to elucidate some of the DMPK properties discussed. Nevertheless, this chapter is important for mass spectrometry practitioners to understand the key factors and relationship between a compound structure and its DMPK properties. DMPK properties defined and explained include oral dose bioavailability, half-life, fraction absorbed, clearance, and volume of distribution. In separate sections, the later three properties are further elaborated. An important message conveyed is that about 40, 30, 20, and 10% of the drug candidates fail owing to poor pharmacokinetics properties, poor clinical efficacy, toxicity, and other unspecified causes, respectively. These compound

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attrition trends have made the pharmaceutical industry recognize that it is necessary to weed out the compounds with poor DMPK properties early in the drug development program and follow the motto "fail fast, but cheap." The author has done an excellent job in describing how pharmaceutical scientists are adhering to this motto by inventing and adapting innovative mass spectrometry methods with high accuracy, enhanced sensitivity, and higher throughput over traditional techniques.

In Chapter 3 "High Throughput Strategies for In Vitro ADME Assays: How Fast Can We Go?," Daniel B. Kassel starts by presenting biopharmaceutical properties of an ideal development drug candidate and, to assist the reader, a tabulated list of these properties. This chapter further points out that to select compounds with desirable biopharmaceutical properties, it is important to implement studies that assess absorption, distribution, metabolism, and excretion (ADME) properties of compounds early in the drug discovery program. Among the ADME properties, absorption and metabolism properties have been identified as key properties to profile at earlier stages of drug discovery. In vitro based screens for metabolic stability, cytochrome P450 inhibition, cell permeability, solubility, plasma stability, and plasma protein binding are important attributes to weed out compounds that are metabolized rapidly and to select or rank compounds for in vivo evaluations.

A large portion of ADME assays are now being incorporated into drug discovery programs in a high throughput mode because they have enhanced sensitivity, selectivity, and ease of automation that is available with LC-MS and LC-MS/MS in comparison with traditional analytical methods (LC-UV, florescence, NMR, etc.). In the next sections, high throughput ADME assays are divided into serial, semi-parallel, and parallel approaches and discussed in detail. In the serial mode of operation, single autosampler/HPLC column, and MS systems are used and samples are injected one at a time, the strategies used to increase throughput include fast chromatography, automated data processing, and pooling strategies (cassette dosing, pooling after individual dosing, simple sample screens, etc.). In semi-parallel or staggered parallel mode of operation, common delays associated with sample loading and HPLC column equilibration times are reduced by using two autosamplers and columns in parallel with a single triple quadrupole mass spectrometer. Parallel LC-MS methods used to support high throughput ADME assay are devised either using nonindexed or indexed mass spectrometry. The general high throughput parallel LC-MS system consists of a high-pressure binary solvent delivery system, a multiple probe autosampler, a switching valve, and a mass spectrometer. Multiple samples (8 or 4) are injected into multiple injection ports (8 or 4) simultaneously and delivered to multiple microbore columns (8 or 4). The HPLC effluent from all columns is delivered to a single mass spectrometer via either single (nonindexed) or multiplexed (indexed or MUX)-ESI source. By using parallel LC-MS approaches, as many as 5000 metabolic stability samples have been analyzed in a single day. Advantages and limitations associated with serial, semi-parallel, and parallel high throughput strategies are well presented, and the reader will learn how automated sample preparation, data acquisition, and data processing have enabled the pharmaceutical industry to move most of the ADME studies into the high throughput realm.

Chapter 4 "Matrix Effects: Causes and Solutions" by Hong Mei is one of the most important chapters for any new comer to the mass spectrometry field. This chapter focuses on providing the reader with an understanding of the ESI process. The ion-evaporation and equilibrium-partitioning models, which are used to predict the ESI response of an analyte based on the properties and concentrations of the analyte and coeluting components, are well presented. In a nutshell, this chapter discusses the possible causes of matrix effects by using the mechanism of ion formation in electrospray. Various approaches for evaluating matrix effects as well as strategies for overcoming matrix effects are also discussed.

Chapter 5 "Direct Plasma Analysis Systems" by Yunsheng Hsieh deals with on-line sample preparation techniques that improve LC-MS and LC-MS/MS throughput by eliminating the bottleneck associated with off-line sample preparation. By using on-line solid-phase extraction (SPE) procedures, analytes are delivered directly from a SPE cartridge into a mass spectrometer. The steps associated with off-line sample preparation (extraction, elution, evaporation, and reconstitution) are thus eliminated.

The application of mass spectrometry for the analysis of acyl glucuronide conjugates is the topic of Chapter 6 "Acyl Glucuronides: Assays and Issues" by Sam Wainhaus. This chapter begins by describing how acyl glucuronides are formed, and once formed how these metabolites can undergo acyl migration, and subsequently bind to protein and trigger immunotoxic reactions. The following section presents several approaches that can be used to assess the toxic nature of acyl glucuronides including mass spectrometry based methods. The next sections show how LC-MS and LC-MS/MS based methodologies can be used to quantify acyl glucuronide formation, assess the extent of acyl migration, and determine protein-adduct formation. This chapter also details special conditions that are used to collect and preserve acyl glucuronides and slow down acyl migrations. This chapter clearly points out that if appropriate sample collection and storage conditions are not used, acyl glucuronides can undergo hydrolysis and result in underestimation of acyl glucuronide metabolites and overestimation of its aglycone concentrations. The reader is also presented with an acyl glucuronide risk

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assessment cube that shows the relationship between immunotoxicity response and LC-MS/MS measured parameters such as acyl glucuronide reactivity, plasma level, and percent conversion.

In chapter 7 "Utilizing Higher Mass Resolution in Quantitative Assays", Xiaoying Xu discusses the new mass spectrometry technology that can provide higher mass resolution for quantitative assays. In particular, triple quadrupole mass spectrometers with enhanced mass resolving capabilities and quadrupole time-of-flight (Q-TOF) mass spectrometers are compared and contrasted for PK applications. Traditionally, separation of an analyte from coeluting matrix components and/or metabolites was achieved by modifying the chromatographic conditions. However, HPLC method modifications come at the expense of time- and resource-consumption and most often end up being methods with longer run times, which are usually not suitable for high throughput quantitative assays. The author shows several examples where utilization of higher mass resolution in quantitative assays is advantageous over modifying chromatographic conditions, changing ionization methods, selecting different MRM or SRM ion pairs, changing different mass spectrometers, or changing the sample processing/clean-up methods.

Chapter 8 "Special Requirements for Metabolite Characterization", authored by Kathleen Cox, reviews the challenges involved in selecting the appropriate mass spectrometry tools and techniques to get information on the metabolic fate of drug candidates in the high-throughput drug discovery environment. A clear distinction is established between high throughput based LC-MS/MS assays used for obtaining absorption, enzyme inhibition, induction, and other pharmacokinetic parameters and the complex, low-throughput process associated with elucidating biotransformation pathways of drug candidates. The reader is introduced to the importance of determining reactive and toxic metabolic liabilities of drug candidates and the necessity of conducting metabolic profiling studies in several species to select appropriate species for toxicological evaluation of drug candidates. Until recently, the complexity, availability of limited supply of test compounds, and unavailability of radiolabeled and/or stable labeled drug candidates relegated metabolite profiling experiments to the development phase of drug development. This paradigm changed with the introduction of atmospheric pressure ionization techniques (ESI, APCI, and APPI) that allowed practical coupling of HPLCs with mass spectrometers. This new technology has paved the way for introduction of a plethora of sensitive and selective quadrupole, ion trap, time-of-flight, and hybrid mass spectrometers that can be used for metabolite characterization. A section dedicated to recent advances in mass spectrometry technology reviews the necessity of using complementary MS instrumentation to understand completely the metabolic fate of drug candidates. Dr. Cox discusses why triple quadrupole mass spectrometers are useful in providing a first look at metabolite profiles of drug candidates, and why ion trap mass spectrometers are necessary to perform sequential MS/MS experiments to narrow the potential sites of modification and provide more complete structural information for metabolites. This section concludes with the discussion of recent advances in the accurate mass and high-resolution mass spectrometers and their utility in the identification of metabolites.

Next, a short section is dedicated to sample preparation and discusses the difficulties associated with developing sample preparation/clean-up methods when the metabolic pathways of drug candidates are unknown. The liquid chromatography (LC) section discusses the importance of separating the metabolites from matrix ions and points out the risks associated with adopting high-throughput LC methods for metabolite profiling experiments. These risks can be overcome by utilizing fast scanning quadrupole time-of-flight mass spectrometers to perform metabolite identification (ID) studies. Advantages associated with utilizing monolithic columns and the merits of turbulent flow chromatography for metabolite profiling experiments are also discussed briefly.

The section, covering the various metabolite ID software programs, discusses the typical software decision tree used for analyzing metabolite characterization study data. MS vendor-supplied software is capable of interrogating LC-MS data, subtracting matrix ions from the mass chromatogram, looking for expected metabolites or characteristic isotopic patterns, providing a list with potential metabolites, and setting up MS/MS experiments to confirm the identity of the metabolites. Although scientist involvement is crucial for metabolite characterization and data interpretation, metabolite ID software can assist to increase dramatically the throughput of discovery metabolite ID studies. The current uses and technology section discusses a tiered approach to metabolite characterization and outlines the types of metabolism experiments and mass spectrometry experiments that are suitable at various stages of drug development. This is a must-read chapter for all scientists who want to embark on metabolite identification studies.

In Chapter 9 "APPI: A New Ionization Source for LC and MS/MS Assays", Yunsheng Hsieh introduces the reader to atmospheric pressure photoionization (APPI) technology. Before introducing the reader to APPI, sections on early and recent development in HPLC-MS interfaces briefly cover various ionization techniques including thermospray, particle beam, continuous-flow fast atom bombardment, sonic spray ionization, ESI, and APCI. The main focus of the chapter is the APPI technique and its application to low-polarity analytes. This chapter also includes a well-presented section on the dopant-assisted APPI, and a section that focuses on techniques used in

evaluating matrix ionization suppression in APPI. Overall, APPI capability is a must for a bioanalytical tool box for tackling low-polarity analytes.

Chapter 10 "Q-Trap MS: A New Tool for Metabolite Identification" by Gerard Hopfgartner and Manfred Zell focuses on the application of hybrid triple quadrupole linear ion trap (QqLIT or Q-Trap) mass spectrometer for drug metabolism studies. Overall, Q-Trap is a versatile mass spectrometer that is suitable for all types of small molecule drug metabolism and pharmacokinetics applications. Using two pharmaceutical compounds, tolcapone and Compound A, the authors compare qualitative and quantitative capabilities of Q-Trap and contrast with those of three-dimensional (3-D) trap, triple quadrupole, and Q-TOF mass spectrometers. Examples show that in comparison to 3-D traps, use of a quadrupole as a linear ion trap significantly enhances the ion-trap performance by increasing ion capacity, improving injection and trapping efficiencies, and increasing duty cycle. The low mass cut-off issues associated with 3-D ion trap and how this limitation is overcome in linear ion traps by using a quadrupole as a collision cell are also covered. For quantitative experiments, some of the nonlinearity associated with space-charge effects, are eliminated by the increased ion capacity of the quadrupole collision cell and the incorporation of dynamic fill time (DFT). Furthermore, fast duty cycle provides the capability to perform as many as 50 SRM transitions within a second. Versatility of the Q-Trap mass spectrometer for elucidating biotransformation pathways, in the absence of a radiolabeled drug, is also demonstrated by using various scan types. To guide the reader, various Q-Trap scans and various information dependent acquisition (IDA) scan combinations are tabulated and discussed as they are applied to the characterization of the metabolites of Compound A, tolcapone, and various other literature examples. Q-Trap technology is a must for drug metabolism laboratories for enabling scientists to make qualitative and quantitative project decisions with confidence.

Chapter 11 "MS Imaging: New Technology Provides New Opportunities" authored by Michelle Reyzer and Richard Caprioli describes the use of mass spectrometry for imaging. Secondary ion mass spectrometry (SIMS), which has been in use for imaging surfaces for more than 40 years, is discussed briefly. The remainder of the chapter is devoted to matrix assisted laser desorption ionization (MALDI) based imaging of peptide, proteins, drugs, and metabolites. Over the past five years, advances in instrument design, computer speed/control, and imaging software, have brought the MALDI-MS imaging technique out of the academic research laboratories and into the pharmaceutical research laboratories. The two most significant advantages for MALDI-MS imaging of low molecular weight compounds are that intact drugs may be analyzed directly from tissues without the use of isotopic- or radio-label compounds, and metabolites can be differentiated from the parent drug. The limitations associated with MALDI-MS for imaging drugs and metabolites directly from tissue are associated with low mass region MALDI matrix ions. Details on how to overcome some of the matrix interference problems and to prepare tissue samples for MALDI-MS imaging are well presented with examples, pictorial guides, and references. These authors clearly show that, in its current form, MALDI-MS imaging is well suited to determine the localization of drug compounds and their metabolites in whole tissue sections. MALDI-MS imaging is also used in screens for receptor selectivities of drug candidates without the use of radiolabeled compounds. This technique is not yet ready for determining subcellular localization of drugs and metabolites due to decreased sensitivity when operating at submicron range resolutions. However, with improvements in MS technology, laser technology, and imaging software, applications of MALDI-MS imaging will indeed increase.

In Chapter 12 "Understanding the Role and Potential of Infusion Nanoelectrospray Ionization for Pharmaceutical Bioanalysis", Bradley Ackerman and Jean-Marie Dethy provide an excellent overview of the automated chip-based infusion nanoelectrospray ionization (nanoESI) technology and its applications to in vitro and in vivo ADME studies. The reader is introduced to the microfabricated silicon chip based ESI source (Nanomate) ion formation process as well as to the inner workings of the source through the use of excellent descriptions and illustrations. A major portion of the chapter is devoted to application of the technology to: (1) non-GLP mode quantification of analytes in plasma, (2) Caco-2 permeation screening, (3) metabolic-stability studies, and (4) GLP mode quantification of analytes in plasma. With each application, advantages and limitations of nanoESI-MS are compared with those of conventional LC-MS/MS methods. A wealth of information is presented on the sample preparation techniques for nanoESI bioanalysis as well as less susceptibility of the nanoESI source to ion suppression compared to ESI at conventional flow rates. Limitations of using the Nanomate 100 for bioanalysis include nozzle-to-nozzle failure rate, inability to control the sample plate temperature, limited array size (10×10), and inability to perform on-line chromatography. Since the completion of the book chapter, Advion Biosciences (Ithaca, NY) introduced a new nanoESI source called "Triversa Nanomate" and improved the software controlling the source to alleviate most of the limitations discussed in this chapter. The new improved source has the capabilities to do nano flow infusion ESI, LC-nano flow ESI, and fraction collection. Furthermore, the array size of the chip has been standardized to 20×20, and the nozzle-to-nozzle reproducibility in-

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creased by using software to sense when the ion current falls below a set threshold and automatically switch over to the next nozzle. The added features of the Advion's nanoESI source make this technology important not only for the pharmaceutical ADME applications described in this chapter but also for metabolite identification, impurity/degradant identification, metabonomics, and proteomics. This chapter is a great starting place for scientists who would want to learn and benefit from the nanoESI technology.

In summary, the book is a valuable addition to the library of anyone working in the ADME area. Chemists, biochemists, or pharmaceutical scientists who wish to obtain an overview of MS applications in drug metabolism studies should consult this book. In the first eight chapters, the editor provides a very useful overview of the application of mass spectrometric methods to a wide variety of qualitative and quantitative drug metabolism properties. Different drug metabolism concepts and their importance in a drug discovery environment are also covered. In the last four chapters, state-of-the-art mass spectrometry technology that can impact discovery and development of new medicines are introduced. Each chapter can be read as a stand-alone chapter, and the reader is directed to the appropriate chapter of interest by a fourteen page index. A guide to abbreviations would have been a useful tool for newcomer. Overall, the production and editing components of this book are presented in a very professional manner without any bias toward any applications, methods, or instrumentations. In addition to being well written, the book is appropriately priced for academic, governmental, and the pharmaceutical scientists.